federal register

Wednesday
April 11, 1979

Part II

Department of Health, Education, and Welfare

National Institutes of Health

Recombinant DNA Research

Actions Under Guidelines

DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE

National Institutes of Health

Recombinant DNA Research; Actions Under Guidelines

AGENCY: National Insitutes of Health, PHS, HEW.

ACTION: Notice of actions under NIH Guidelines for Research Involving Recombinant DNA Molecules.

SUMMARY: This notice sets forth actions taken by the Director, NIH, under the 1978 NIH Guidelines for Research Involving Recombinant DNA Molecules (43 FR 60108).

EFFECTIVE DATE: April 11, 1979.

FOR FURTHER INFORMATION CONTACT: Additional information can be obtained from Dr. William J. Gartland, Office of Recombinant DNA Activities, National Institutes of Health, Bethesda, Maryland 20205, (301) 496-6051.

SUPPLEMENTARY INFORMATION: I am promulgating today several major actions under the NIH Guidelines for Research Involving Recombinant DNA Molecules. These proposed actions were published for comment in the **Federal Register** of January 15, 1979 and reviewed and recommended for approval by the Recombinant DNA Advisory Committee (RAC) at its meeting on February 15–16, 1979. In accordance with Section IV–E–1–b of the NIH Guidelines, I find that these actions comply with the Guidelines and present no significant risk to health or the environment.

Part I of this announcement provides background information on the actions. Part II lists the major actions.

I. Decisions on Actions Under Guidelines

A. *Lower Eukaryotes as Host-Vector Systems*

Consideration of the use of lower eukaryotes as host-vector systems was initiated at a meeting at NIH of a Working Group on Lower Eukaryote Host-vector Systems on September 16, 1978. The purpose of the meeting was to consider possible alternatives to the EK1 and EK2 systems. Based on the routes of escape, the possibility of mating with naturally occurring strains (and, therefore, potential transfer of cloned material) and the ecological niches available to *Saccharomyces cerevisiae* and *Neurospora crassa*, it was reasoned that considerable containment is afforded by normal laboratory strains of *S. cerevisiae* and mutationally modified strains of *N. crassa*. In addition, it was unanimously agreed that *S. cerevisiae* could meet the criteria of HV2 systems if transfer of cloned DNA was prevented by mutations that would prevent mating. A Working Group report was developed from this meeting and presented to the Recombinant DNA Advisory Committee (RAC) at its October 30–31, 1978 meeting. The report was modified and approved by the RAC. The recommendations were summarized and published for comment in the **Federal Register** on January 15, 1979. No comments were received during the 30-day period for comment. The RAC again considered the recommendations at its meeting on February 15–16, 1979.

1. *N. CRASSA* AND *S. CEREVISIAE* AS HV1 SYSTEMS. The RAC considered the following proposal for approval of *Neurospora crassa* and *Saccharomyces cerevisiae* as HV1 systems:

"*N. crassa* and *S. cerevisiae* as HV1 systems. Specified strains of *N. crassa* which have been modified to prevent aerial dispersion and unmodified laboratory strains of *S. cerevisiae* are acceptable as HV1 systems based on their natural containment. The following *N. crassa* strains can be used:

(1) inl (inositolless) strains 37102, 37401, 46316, 64001 and 89601.

(2) csp-1 strain UCLA37 and csp-2 strains FS 590, UCLA101 (these are conidial separation mutants)

(3) eas strain UCLA191 (an "easily wettable" mutant)."

At the February 15–16, 1979 RAC meeting, the report was summarized and some of the advantages of using the lower eukaryote host-vector systems were described. The organisms are structurally and genetically more related to the cells of higher eukaryotes and there is the possibility of obtaining gene expression that may not occur in *E. coli*, particularly due to possibility of processing intervening sequences. The extensive knowledge of the genetics of *Saccharomyces* and *Neurospora* will enable significant contributions by investigators who have worked extensively with these organisms.

It was explained that the basis for proposing these organisms for HV1 containment came from considerations of the modes of escape, the opportunity for establishment in nature, and the possibility of transferring cloned segments to other species or strains in nature. As discussed in the report, there is very little opportunity to mate with other strains or species in nature and, furthermore, there are no natural vectors known which might effect interspecific transfer or genetic material.

Saccharomyces cerevisiae and *Neurospora crassa* are nonpathogenic to man, animals, and plants, and not closely related to known pathogens. Therefore, the RAC feels that relevant and important experiments can be done safely without risk to man, animals, plants or the general environment. The ecological niche for *S. cerevisiae* is the surface of fruits or the nectar of flowers. *N. crassa* is found abundantly at barren, burned-out sites in the semi-tropics. Neither organism can colonize the gut of man. Large amounts of bread and beer are consumed without any pathogenic effects of the yeast that are present.

The RAC voted to accept this section of the report by a vote of 19 to 0 with 1 abstention.

2. USE OF UNMODIFIED LABORATORY STRAINS OF *N. CRASSA*. The RAC considered the following proposal for use of unmodified laboratory strains of *N. crassa:*

"Unmodified laboratory strains of *N. crassa* can be used in experiments that require the HV1 level of containment provided that these are carried out at physical containment one level higher than required for HV1. However, if P3 physical containment is specified for HV1, this level is considered adequate for unmodified *N. crassa*. For P2 physical containment, special care must be exercised to prevent aerial dispersal of macroconidia."

This action is essentially equivalent to allowing the use of *N. crassa* as an "HV0" system. The scientific reason for proposing this is that a wide variety of mutants are available in *N. crassa*. The restriction of experiments to a few strains would require considerable genetic manipulation and effort which could be avoided by use of unmodified laboratory strains.

Several RAC members were concerned about the escape of *Neurospora* in aerosols or into the air since it forms spores which are freely dispersed. Since the intent was to have investigators exercise caution in their use of wild-type strains, it was proposed that the original proposed action be made more specific by the addition of the statement "including the use of a biological safety cabinet" after the last word in the paragraph. The RAC voted 18 to 1 with 1 abstention to accept this section as amended.

However, later in the discussion on the equivalence of lower eukaryote HV systems with those of *E. coli*, the RAC voted 18 to 0 with 2 abstentions that unmodified laboratory strains of *N. crassa* be approved at the present time

only for certain experiments. Accordingly, this section will now read as follows:

"Unmodified laboratory strains of *N. crassa* are approved at the P3 level of containment for shotgun experiments with phages, plasmids, and DNA from Class 1 prokaryotes [1] and lower eukaryotes that do not produce polypeptide toxins. [34]"

3. *S. CEREVISIAE* AS AN HV2 SYSTEM. The RAC considered the following criteria for the use of *S. cerevisiae* as an HV2 system:

"*S. cerevisiae* as an HV2 system. Biological containment of yeast is a product of several factors: (a) probability of encounter of the host-vector with a wild type yeast outside the laboratory; (b) the abundance of wild type strains able to mate with a laboratory strain; (c) the frequency of mating under the dilute conditions simulating natural environments; (d) the reduction in mating fequency conferred by the sterility mutations; (e) the stability of the cloned segment and its vector; (f) the survival and growth ability of the host relative to wild types.

The following data are to be supplied in support of a candidate *S. cerevisiae* for HV2 certification:

(1) Genotype of the strain, description of the vectors and selective markers to be used, the nature and stability of the mutations(s) contributing to sterility and the mode of construction of the strain.

(2) The frequency of mating (in a worst case analysis under optimal conditions) with a fertile strain of opposite mating type.

(3) Relative growth rates of the candidate strain and suitable industrial wild types, separately and in mixed culture. (The specification of industrial strains reflects the rarity of *S. cerevisiae* in nature under non-domesticated conditions.)

(4) Data on the stability and maintenance of the cloned segment and vector in nonselective media.

(5) Measurement of the relative ability (in a worst case analysis) of fertile parents of the sterile HV2 candidate to mate at low cell density compared to optimal conditions.

(6) An experimental estimate of the frequency of transfer of a model cloned segment (in a worst case analysis, again with a fertile strain) to a non-mating, industrial strain, after prolonged cultivation in mixed culture.

Changes of auxotrophic selective markers in host and vector, or change in mating type allele, are trivial modifications not requiring certification, provided the level of sterility is not decreased. Certified EK2 *E. coli* vector DNA or fragments thereof can be used in HV2 *S. cerevisiae*."

This section addresses the criteria for the acceptance of *S. cerevisiae* as an HV2 system. One member of the RAC raised a question as to whether numerical values should be assigned to the containment criteria. It was stated that the combined effect of various containing features (the product) is the important consideration. Explicit in the Guidelines is the factor of 10^{-8} (See Guidelines, Section II–D–1–b). Investigators will have to present data that will at least satisfy this value.

The RAC voted to accept this section by a vote of 19 to 1 with no abstentions.

4. EQUIVALENCE OF LOWER EUKARYOTE HV SYSTEMS WITH THOSE OF *E. COLI*. The RAC considered a proposal for the equivalence of lower eukaryote HV systems with those of *E. coli*. The proposal appeared in the January 15, 1979 **Federal Register** as follows:

"Equivalence of lower eukaryote HV systems with those of *E. coli*. In accord with Section III–B–1, host-vector systems which have been approved as HV1 systems may be used under P2 containment conditions for shotgun experiments with phages, plasmids, and DNA from nonpathogenic prokaryotes which do not produce polypeptide toxins. [34] For other classes of recombinant DNA experiments with these HV1 systems, except for the cloning of complete genomes of eukaryote viruses, the *S. cerevisiae* and *N. crassa* HV1 systems and *S. cerevisiae* HV2 systems may be used at the physical containment levels applicable to EK1 and EK2 systems, respectively. Experiments involving complete genomes of class 1 eukaryote viruses will require P3+HV2 containment levels. Other eukaryote viruses are to be handled on a case-by-case basis. [45]"

This section contained a typographic error as it appeared in the **Federal Register** of January 15, 1979. The next to last sentence in the paragraph should have read:

"Experiments involving complete genomes of class 1 eukaryote viruses will require P3+HV1 or P2+HV2 containment levels."

The RAC decided to treat this proposal in two sections. Accordingly, the RAC first considered the following part of the proposal:

"Equivalence of lower eukaryote HV systems with those of *E. coli*. In accord with Section III–B–1, host-vector systems which have been approved as HV1 systems may be used under P2 containment conditions for shotgun experiments with phages, plasmids, and DNA from nonpathogenic prokaryotes which do not produce polypeptide toxins. [34] For other classes of recombinant DNA experiments with these HV1 systems, except for the cloning of complete genomes of eukaryote viruses, the *S. cerevisiae* and *N. crassa* HV1 sysems and *S. cerevisiae* HV2 systems may be used at the physical containment levels applicable to EK1 and EK2 systems, respectively."

In order to use the approved systems in various experiments it is necessary to assign containment levels. One RAC member pointed out that if equivalence was not adopted it would be necessary to go through the Guidelines and individually assign containment levels for each set of experiments.

The RAC proposed to equate HV1 to EK1 and HV2 to EK2 for the *S. cerevisiae* and *N. crassa* systems.

The RAC voted to accept the proposal by a vote of 18 to 2 with no abstentions.

The RAC then considered the second part of this section which was incorrectly printed in the **Federal Register** of January 15, 1979. The RAC in its deliberations took a less restricted position than either that of the **Federal Register** announcement or the report of the Working Group, and recommend that the following wording be substituted for the previous proposal:

"Experiments involving complete genomes of eukaryote viruses will require P3+HV1 or P2+HV2 containment levels."

This recommendation passed by a vote of 17 to 1 with 2 abstentions. Since this is a major change in the proposed action as it appeared in the **Federal Register** on January 15, 1979, additional opportunity for public comment is appropriate. Accordingly, this proposed action will be published for an additional 30-day comment period and will be reconsidered by the RAC at its next meeting.

B. *Saccharomyces Cerevisiae* HV2 Systems

The RAC reviewed an application submitted by Dr. David Botstein et al. for certification of sterile yeast hosts (SHY strains of *Saccharomyces cerevisiae)* as HV2 containment systems when used with a variety of composite vectors capable of replication and/or integration in yeast.

During the 30-day period for comment, no comments were received. Prior to consideration of this proposal, the RAC had recommended the criteria for *S. cerevisiae* as an HV2 system described in A above.

The application had been reviewed by the Lower Eukaryotic Host-Vector Working Group and recommended to the RAC for acceptance. The basis of containment in the HV2 systems is the inability of cloned DNA in a sterile yeast host to be transferred out of the host to other laboratory or wild type strains. The level of containment achieved by the HV2 system is acceptable based primarily upon the sterility, the probability of encountering an appropriate haploid strain in nature which is capable of mating, and the inefficiency of mating in dilute suspension.

The following sterile strains of yeast, all of which have the *ste*-VC9 mutation, are proposed as HV2 hosts: SHY1, SHY2, SHY3 and SHY4.

The proposed HV2 plasmid vectors have in common several characteristics. They all contain a bacterial replicon derived from the certified EK2 vector, pBR322, which makes possible the amplification of the DNA in *E. coli*. Another common characteristic is the presence of markers which enable selection in *E. coli* for the presence of the plasmid. Some of these markers are drug-resistance genes derived from pBR322. Others, however, are yeast DNA fragments which are expressed in *E. coli* K–12: these include the *ura*3, *leu*2, *his*3, and *trp*l genes.

Most of the vectors also carry one or more genes of yeast origin which can be used to select the presence of the vector in yeast, such as *ura*3, *leu*2, *trp*1, and *his*3. The SHY strains are arranged to make any or all of these genes selectable. All the vectors also carry fragments of yeast DNA which provide for the maintenance of the plasmid in yeast. The following are the proposed HV2 plasmid vectors: YIp1, YEp2, YEp4, Ylp5, YEp6, YRp7, YEp20, YEp21, YEp24, Ylp25, Ylp26, Ylp27, Ylp28, Ylp29, Ylp30, Ylp31, Ylp32, Ylp33.

A complete discussion of the strains and the vectors proposed for certification is available from the Office of Recombinant DNA Activities.

The RAC voted 17 to 0 with 3 abstentions to recommend approval of the proposed systems because they were considered to meet adequately the criteria for HV2 *S. cerevisiae* systems.

C. *Prokaryots Other than E. Coli as Host-Vector Systems*

This issue was considered at a meeting held at Stanford University on September 12, 1978 of the Working Group on Prokaryote Host-Vectors Other than *E. coli*. The purpose of that meeting was to explore other candidate prokaryotic organisms as HV1 systems. The group considered *Bacillus* and *Streptomyces* systems as possible additions to the presently approved EK1 and EK2 systems. The Working Group report was considered by the RAC at its October 30–31, 1978 meeting. The report was modified and approved by the RAC. The recommendations were summarized and published for public comment in the **Federal Register** on January 15, 1979. No comments were received during the 30-day period for comment. The RAC again considered the recommendations at its meeting on February 15–16, 1979.

1. EXEMPTION OF *BACILLUS* SPECIES THAT EXCHANGE GENETIC INFORMATION. The RAC considered the following proposal for exemption of certain *Bacillus* species on the basis of exchange of genetic information:

"*Bacillus subtilis* related species that have been shown to exchange chromosomal DNA will be included under the exemption category of Section I–E–4 of the 1978 Guidelines. Any recombinant DNA molecules that are composed entirely of DNA segments from one or more of the organisms listed below and to be propagated in any of the organisms listed below are exempt from the Guidelines. (This list is to be separate from that which already exists in Appendix A for exchangers with *E. coli.)*

Bacillus subtilis
Bacillus licheniformis
Bacillus globigii
Bacillus niger
Bacillus nato
Bacillus pumilus
Bacillus amyloliquefaciens
Bacillus aterrimus"

The basis of this list is that these organisms exchange chromosomal DNA by transformation.

The RAC voted 17 to 0 with 3 abstentions to accept this section of the report.

2. *BACILLUS SUBTILIS* HV1 SYSTEM. The RAC considered the following proposal for acceptance of *B. subtilis* as an HV1 system:

"Asporogenic mutant derivatives of *B. subtilis* can be accepted as the host component of an HV1 system. These derivatives must not revert to sporeformers with a frequency greater than 10^{-7}; data confirming this requirement must be presented to NIH for certification. The following plasmids are accepted as the vector components of certified *B. subtilis* HV1 systems: pUB110, pC194, pS194, pSA2100, pE194, pT127, pUB112, pC221, pC223."

The *Bacillus subtilis* system was recommended by the Working Group as a host system in conjunction with certain plasmids because it offers distinct advantages for genetic manipulation and is of little or no potential hazard. *B. subtilis*, an obligate aerobe, is not normally indigenous to man, is non-pathogenic to man, and is generally found in the soil. In Japan, a vegetable cheese composed of live *Bacillus nato*, almost identical to *B. subtilis*, is consumed in large quantities by an estimated 80% of the population. In France, deliberate ingestion of *B. subtilis* known as "Bactisubtil" is similarly widespread and is used therapeutically for certain digestive disorders. *B. subtilis* is not a normal inhabitant of the gut since it is an obligative aerobe.

The RAC voted 15 to 0 with 4 abstentions to approve the proposal.

3. EQUIVALENCE OF *BACILLUS SUBTILIS* HV1 SYSTEMS WITH THOSE OF *E. COLI*. The following statement regarding equivalency of *Bacillus subtilis* systems that had been approved by the RAC at its meeting on October 15–16, 1978 was omitted from the January 15, 1979 **Federal Register** announcement:

"HV1 *Bacillus subtilis* systems will be equivalent to EK1 in the physical containment requirements for specific experiments."

Section III–B–1 of the Guidelines states:

"Host-vector systems which have been approved as HV1 systems may be used under P2 containment conditions for shotgun experiments with phages, plasmids, and DNA from nonpathogenic prokaryotes which do not produce polypeptide toxins. [34] Other classes of recombinant DNA experiments with these HV1 systems will require prior approval and classification by NIH.

The RAC voted 12 to 0 with 6 abstentions that classes of recombinant DNA experiments with *B. subtilis* HV1 systems (other than those specified above) will be equivalent to EK1 in the physical containment requirements for specific experiments.

4. ADDITION TO SECTION III–B–3, NON–HV1 SYSTEMS. The RAC considered a proposal in the Working Group report to amend Section III–B–3 of the Guidelines by addition of the following:

"In those cases where genetic exchange has not been demonstrated between two bacterial species A and B, neither of which is pathogenic for man, animals, or plants, recombinant DNA experiments involving only A and B can be conducted under P3 containment.[2A]"

The RAC voted 19 to 1 to accept an amended statement which reads as follows:

"In those cases where genetic exchange has not been demonstrated between two bacterial species A and B, neither of which is known to be pathogenic for man, animals, or plants, recombinant DNA experiments involving only A and B can be conducted under P3 containment.[2A]"

D. *Specific Bacillus Subtilis Experiment.* The RAC considered a request to permit cloning in *B. subtilis*, under P2 conditions, of DNA derived from either *E. coli* K–12 or *Saccharomyces cerevisiae* using NIH-approved EK2 plasmid vectors. No comments were received during the 30-day period for comment.

The RAC agreed that propagation in *B. subtilis* of *E. coli* K–12 DNA on an *E. coli* vector would be an exempt experiment under Section I–E–3. The RAC passed a motion by a vote of 11 to 0 with 2 abstentions to permit the cloning in *B. subtilis*, under P2 conditions, of DNA derived from *Saccharomyces cerevisiae* using EK2 plasmid vectors provided that an HV1 *B. subtilis* host is used.

E. *Containment Levels for Shotgun Cloning of Primate and Other Mammalian DNA in E. Coli K–12*

The RAC considered proposed changes in the Sections III–A–1–a–(1) and –(2) of the Guidelines so as to include the possibility of P3+EK1 containment as an alternative to the present P2+EK2 containment requirement for the shotgun cloning of primate or other mammalian DNA into *E. coli* K–12. During the 30-day period for public response, no comments were received.

This proposal had been considered during the revision of the Guidelines and the issue is discussed in the Decision Document which accompanied the Revised Guidelines (**Federal Register**, December 22, 1978, page 60088).

The RAC considered the possibility of requiring an EK2 certified vector in EK1 hosts or even a "nonmobilizable vector" as had been proposed at the last RAC meeting. Two members emphasized that there should be no greater containment required for general cloning of primate or mammalian DNA than is already required for tumor viruses. One member described at length the physical containment provided by a P3 facility which under normal medical microbiological laboratory conditions is used only for serious pathogens.

The RAC voted 16 to 1 with 4 abstentions to amend Sections III–A–1–a–(1) and III–A–1–a–(2) of the Guidelines to read as follows:

"III–A–1–a–(1). *Primates.* P2 physical containment+an EK2 host-vector or P3+EK1. Any lowering of containment below these levels (i.e., for purified DNA or characterized clones) cannot be made solely by an institutional biosafety committee but requires NIH approval. (See Section IV–E–1–b–(3).)

III–A–1–a–(2). *Other Mammals.* P2 physical containment+an EK2 host-vector or P3+EK1."

The RAC also indicated its desire that these containment levels for EK1 systems should apply to other HV1 systems under equivalence provisions, discussed above.

F. *Amendment of Sections III–B–2 and III–C–5 of the Guidelines*

The RAC considered for approval changes in the Sections III–B–2 and III–C–5 of the Guidelines so as to include the words "into a lambdoid phage vector." This change had been previously recommended by the RAC at its October 1978 meeting. During the 30-day period for public response, no comments were received.

In the publication of proposed changes in the **Federal Register** on January 15, 1979, it was noted that the greated flexibility afforded with non-EK2 lambdoid phage vectors does not in any way compromise safety or containment in the final host. The continued requirement for EK2 plasmid vectors insures that the final cloning back into the host of origin can be done with a minimal introduction of *E. coli* genetic information.

It was emphasized that the subject of these sections is essentially the self-cloning of DNA with the introduction of either lambda or *E. coli* plasmid DNA. For the case of plasmid vectors there might be the possibility of the vector carrying some *E. coli* chromosomal DNA. This situation is less likely for the lambda bacteriophage vectors.

The RAC voted 19 to 0 with 2 absentions to amend Sections III–B–2 and III–C–5 of the Guidelines to read as follows:

"Section III–B–2. *Return of DNA segment to Non-HV1 host of origin* * * * * * For a prokaryote which does not exchange genetic information with *E. coli* (Host B), the following type of experiment may be carried out without Host B having been approved as a HV1 host: DNA from Host B may be inserted into a lambdoid phage vector or into a vector from a certified EK2 host-vector system and propagated in *E. coli* K–12 under the appropriate containment conditions [see Section III–A–1–b–(2)]. Subsequently, this recombinant DNA may be returned to Host B and propagated in Host B under P1 conditions. [43]

III–C–5. *Fungal or Similar Lower Eukaryotic Host-Vector Systems* * * * * * * In addition to the experiments described above, the following experiments may be carried out without the eukaryotic host (Host C) having been approved as an HV1 host: DNA from Host C may be inserted into a lambdoid phage vector or into a vector from a certified EK2 host-vector system and propagated in *E. coli* K–12 under the appropriate containment conditions [see Section III–A–1–(a)–(5)]. Subsequently, this recombinant DNA may be returned to Host C and propagated there under P1 conditions. [43] * * *

G. *Amendment of Section III–C–2, Invertebrate Host-Vector Systems*

The RAC recommended that Section III–C–2 concerning *invertebrate host-vector systems* be divided into two parts. The first part, III–C–2–a, would retain the present wording of this section; the second part III–C–2–b would enable the use of nonviral vectors. Section III–C–2 would therefore read as follows:

"III–C–2. *Invertebrate Host-vector systems.*

III–C–2–a. *Insect viral vectors.* As soon as information becomes available on the host range restrictions and on the infectivity, persistence, and integration of the viral DNA invertebrate and invertebrate cells, experiments involving the use of insect viruses to propagate DNA sequences will be evaluated by NIH on a case-by-case basis [45] and will be conducted under the recommended physical containment conditions. (See Section IV–E–1–b–(3)–(c)).

III–C–2–b. *Nonviral vectors.* Organelle, plasmid, and chromosomal DNAs may be used as vectors. DNA recombinants formed between such vectors and host DNA, when propagated only in that host (or a closely related strain of the same species), are exempt from these Guidelines (see section I–E). DNA recombinants formed between such vectors and DNA from cells other than the host species require P1 physical containment for invertebrate cells in culture since invertebrate cells in culture inherently exhibit a very high level of containment. Experiments which require the use of whole animals will be evaluated by NIH on a case-by-case basis [45]."

Section III–C–2–b is being added to permit the use of nonviral vectors in invertebrate host-vector systems. The revised Guidelines permit the use of nonviral vectors for plant host-vector systems, but do not address their use for invertebrate systems. No comments were received during the 30-day period for comment. The RAC voted 12 to 0 with 2 abstentions to recommended the proposed amendment of Section III–C–2.

H. *Addition of Section III–C–1–f, Vertebrate Host-Vector Systems—Non-Viral Vectors*

Similar to the proposal in G above, the RAC recommended that a comparable section be added to Section III–C–1 on vertebrate host-vector systems. The new sub-section would read as follows:

"III–C–1–f. *Nonviral vectors.* Organelle, plasmid and chromosomal DNAs may be used as vectors. DNA recombinants formed between such vectors and host DNA, when propagated only in that host (or a closely related strain of the same species), are exempt from these Guidelines (see Section I–E). DNA recombinants formed between such vectors and nonviral DNA from cells other than the host species require only P1 physical containment for cells in culture since vertebrate cells in tissue culture inherently exhibit a very high level of containment. Recombinants involving viral DNA or experiments which require the use of whole animals will be evaluated by NIH on a case-by-case basis [45]."

No comments were received during the 30-day period for comment. The RAC voted 10 to 0 with 4 abstentions to accept this proposal.

I. *Addition of Section III–C–6, Return of DNA Segments To a Higher Eukaryotic Host of Origin*

The RAC considered the following proposal to add a new Section (III–C–6) to the Guidelines to permit the return of DNA segments to a higher eukaryotic host of origin:

"III–C–6. *Return of DNA segments to a Higher Eukaryotic Host of Origin.* DNA from a higher eukaryote (Host D) may be inserted into a lambdoid phage vector or into a vector from a certified EK2 host-vector system and propagated in *E. coli* K–12 under the appropriate containment conditions [see Section III–A–1]. Subsequently, this recombinant DNA may be returned to Host D and propagated under conditions of physical containment comparable to P1 and appropriate to the organism under study. [2A]"

The RAC noted that the revised Guidelines do not address the question of cloning within a higher eukaryote or cultured cells derived therefrom of DNA taken from the same species, joined to a vector and cloned in *Escherichia coli* K–12, and then returned to cells of the species of origin. Analogous clauses exist for returning DNA from *Escherichia coli* to a species of origin from prokaryotes and lower eukaryotes. The experiment is essentially a "self-cloning" experiment and therefore does not require high levels of physical containment. Most such experiments would be exempt from the Guidelines altogether were it not for the fact that the DNA, when returned to the host of origin, is joined to the DNA of the cloning vector, which is of bacterial origin. These vectors comprise a small number of well-characterized harmless DNA molecules.

The types of physical containment (P1 through P4) defined in the Guidelines are applicable to the propagation of microorganisms and to tissue cultures, but not to whole multicellular organisms. Thus, it is impractical to require P1 containment, and more appropriate to require conditions providing a degree of containment comparable to P1.

The announcement in the January 15, 1979 **Federal Register** drew three letters of comment prior to the February 15–16, 1979 RAC meeting. All three comments requested that the proposal be changed to permit the transfer of DNA segments to a heterologous eukaryote instead of only to the host of origin. The RAC felt that adoption of this proposed change would constitute a major change that would require an additional period for comment.

The RAC voted 13 to 0 with 2 abstentions to accept the proposal as published in the January 15, 1979 **Federal Register** with the following interpretations:

1. the term "host" is interpreted to be "species;"

2. viral DNA from viruses endogenous to a species is considered to be DNA from the species; and

3. this does not include exogenous viruses at this time.

II. MAJOR ACTIONS UNDER GUIDELINES

A. *N. Crassa and S. Cerevisiae as HV1 Systems*

Specified strains of *Neurospora crassa* which have been modified to prevent aerial dispersion, and unmodified laboratory strains of *Saccharomyces cerevisiae* are acceptable as HV1 systems based on their natural containment. The following *N. Crassa* strains can be used:

(1) inl (inositolless) strains 37102, 37401, 46316, 64001 and 89601.

(2) csp-1 strain UCLA37 and csp-2 strains FS 590, UCLA101 (these are conidial separation mutants).

(3) eas strain UCLA191 (an "easily wettable" mutant).

B. *Unmodified Laboratory Strains of N. Crassa*

Unmodified laboratory strains of *N. crassa* are approved at the P3 level of containment for shotgun experiments with phages, plasmids, and DNA from Class 1 prokaryotes [1] and lower eukaryotes that do not produce polypeptide toxins. [34]

C. *Criteria for Saccharomyces Cerevisiae as an HV2 System*

The following are criteria for *Saccharomyces cerevisiae* as an HV2 system. Biological containment of *Saccharomyces cerevisiae* is a product of several factors: (a) probability of encounter of the host-vector with a wild type yeast outside the laboratory; (b) the abundance of wild type strains able to mate with a laboratory strain; (c) the frequency of mating under the dilute conditions simulating natural environments; (d) the reduction in mating frequency conferred by the sterility mutations; (e) the stability of the cloned segment and its vector; (f) the survival and growth ability of the host relative to wild types.

The following data are to be supplied in support of a candidate *S. cerevisiae* for HV2 certification:

(1) Genotype of the strain, description of the vectors and selective markers to be used, the nature and stability of the mutation(s) contributing to sterility and the mode of construction of the strain.

(2) The frequency of mating (in a worst case analysis under optimal conditions) with a fertile strain of opposite mating type.

(3) Relative growth rates of the candidate strain and suitable industrial wild types, separately and in mixed culture. (The specification of industrial strains reflects the rarity of *S. cerevisiae* in nature under non-domesticated conditions.)

(4) Data on the stability and maintenance of the cloned segment and vector in nonselective media.

(5) Measurement of the relative ability (in a worst case analysis) of fertile parents of the sterile HV2 candidate to mate at low cell density compared to optimal conditions.

(6) An experimental estimate of the frequency of transfer of a model cloned

segment (in a worst case analysis, again with a fertile strain) to a nonmating, industrial strain, after prolonged cultivation in mixed culture.

Changes of auxotrophic selective markers in host and vector, or change in mating type allele are trivial modifications not requiring certification, provided the level of sterility is not decreased. Certified EK2 *E. coli* vector DNA or fragments thereof can be used in HV2 *S. cerevisiae.*

D. *Equivalence of Lower Eukaryote HV Systems with those of E. Coli*

In accord with Section III–C–5, host-vector systems which have been approved as HV1 systems may be used under P2 containment conditions for shotgun experiments with phages, plasmids, and DNA from nonpathogenic prokaryotes which do not produce polypeptide toxins. [34] For other classes of recombinant DNA experiments with these HV1 systems, except for the cloning of complete genomes of eukaryote viruses, the *S. cerevisiae* and *N. crassa* HV1 systems and *S. cerevisiae* HV2 systems may be used at the physical containment levels applicable to EK1 and EK2 systems, respectively.

E. *Certified Saccharomyces Cerevisiae HV2 Systems*

The following sterile strains of *Saccharomyces cerevisiae,* all of which have the *ste*-VC9 mutation, are certified as HV2 hosts: SHY1, SHY2, SHY3, and SHY4. The following plasmids are certified for use in an HV2 system: YIp1, YEp2, YEp4, YIp5, YEp6, YRp7, YEp20, YEp21, YEp24, YIp25, YIp26, YIp27, YIp28, YIp29, YIp30, YIp31, Yip32, and YIp33. These plasmids can be considered EK2 vectors when propagated in *x*1776.

F. *Exemption of Bacillus Species that Exchange Genetic Information*

The following *Bacillus subtilis* related species that have been shown to exchange chromosomal DNA are now included under the exemption category of Section I–E–4 of the 1978 Guidelines. Any recombinant DNA molecules that are composed entirely of DNA segments from one or more of the organisms listed below and to be propagated in any of the organisms listed below are exempt from the Guidelines. (This list is to be separate from that which already exists in Appendix A for exchangers with *E. Coli.)*

Bacillus subtilis
Bacillus licheniformis
Bacillus pumilus
Bacillus globigii
Bacillus niger
Bacillus nato
Bacillus amyloliquefaciens
Bacillus aterrimus

G. *Bacillus Subtilis HV1 System*

Asporogenic mutant derivatives of *B. subtilis* can be accepted as the host component of an HV1 system. These derivatives must not revert to sporeformers with a frequency greater than 10^{-7}; data confirming this requirement must be presented to NIH for certification. The following plasmids are accepted as the vector components as certified *B. subtilis* HV1 systems: pUB11o, pC194, pS194, pSA2100, pE194, pT127, pUB112, pC221, pC223.

H. *Equivalence of Bacillus Subtilis HV1 Systems with Those of E. Coli*

Host-vector systems which have been approved as HV1 systems may be used under P2 containment conditions for shotgun experiments with phages, plasmids, and DNA from nonpathogenic prokaryotes which do not produce polypeptide toxins. [34] Other classes of recombinant DNA experiments with *B. subtilis* HV1 systems will be equivalent to EK1 in the physical containment requirements for specific experiments.

I. *Modification of Section III–B–3*

Section III–B–3 of the Guidelines is replaced with the following modified text:

III–B–3. *Non-HV1 Systems.* Containment levels for other classes of experiments involving non-HV1 systems may be approved by the Director, NIH. (See Sections IV–E–1–b–(1)–(b), IV–E–1–b–(2)–(c), and IV–E–1–b–(3)–(b).)

In those cases where genetic exchange has not been demonstrated between two bacterial species A and B, neither of which is known to be pathogenic for man, animals, or plants, recombinant DNA experiments involving only A and B can be conducted under P3 containment. [2A]

J. *Specific Bacillus Subtilis Experiment*

The following experiment has been approved: The cloning in *B. subtilis,* under P2 conditions, of DNA derived from *Saccharomyces cerevisiae* using EK2 plasmid vectors provided that an HVI *B. subtilis* host is used.

K. *Modification of Sections III–A–1–a–(1) and III–A–1–a–(2), Containment Levels for Shotgun Cloning of Primate and Other Mammalian DNA E. Coli K–12*

Sections III–A–1–a–(1) and III–A–1–a–(2) of the Guidelines are replaced with the following modified text:

III–A–1–a–(1). *Primates.* P2 physical containment + an EK2 host-vector or P3 + EK1. Any lowering of containment below these levels (i.e., for purified DNA or characterized clones) cannot be made solely by an institutional biosafety committee but requires NIH approval. (See Section IV–E–1–b–(3).)

III–A–1–a–(2). *Other Mammals.* P2 physical containment + an EK2 host-vector or P3+EK1.

L. *Modification of Section III–B–2*

Section III–B–2 of the Guidelines is replaced with the following modified text:

III–B–2. *Return of DNA Segments to Non-HV1 Host of Origin.* Those prokaryotes that exchange genetic information with *E. coli* by known physiological processes will be exempt from these Guidelines if they appear on the "list of exchangers" set forth in Appendix A (see Section I–E–4). For a prokaryote which can exchange genetic information [35] with *E. coli* under laboratory conditions but which is not on the list (Host A), the following type of experiment may be carried out under P1 conditions without Host A having been approved as an HV1 host: DNA from Host A may be inserted into a vector and propagated in *E. coli* K–12 under P1 conditions. Subsequently, this recombinant DNA may be returned to Host A by mobilization, transformation, or transduction and may then be propagated in Host A in any desired vector under P1 conditions.

For a prokaryote which does not exchange genetic information with *E. coli* (Host B), the following type of experiment may be carried out without Host B having been approved as an HV1 host: DNA from Host B may be inserted into a lambdoid phage vector or into a vector from a certified EK2 host-vector system and propagated in *E. coli* K–12 under the appropriate containment conditions [See Section III–A–1–b–(2)]. Subsequently, this recombinant DNA may be returned to Host B and Propagated in Host B under P1 conditions. [43]

M. *Modification of Section III–C–5*

Section III–C–5 of the Guidelines is replaced with the following modified text:

III–C–5. *Fungal or Similar Lower Eukaryotic Host-Vector Systems.* The containment criteria for DNA recombinant experiments using these host-vectors most closely resemble those for prokaryotes, rather than those for the preceding eukaryotes, since the host cells usually exhibit a capacity for dissemination outside the laboratory

that is similar to that for bacteria. Therefore, the procedures established for certification of HV systems other than *E. coli* K–12 (Section II–D–2) will also apply to these fungal or similar lower eukaryotic host-vector systems.

Once an HV1 system is approved by NIH, it may be used under P2 containment for shotgun experiments with phages, plasmids, and DNA from Class 1 prokaryotes [1] and lower eukaryotes that do not produce polypeptide toxins. [34] Other classes of recombinant DNA experiments with these HV1 systems will require prior approval and classification by NIH. (See Sections IV–E–1–b–(1)–(b), IV–E–1–b–(2)–(c), and IV–E–1–b–(3)–(b).) If HV2 or HV3 systems of this type are developed and approved by NIH, guidelines for their use in other types of recombinant DNA experiments will also be established.

In addition to the experiments described above, the following experiments may be carried out without the eukaryotic host (Host C) having been approved as an HV1 host: DNA from Host C may be inserted into a lambdoid phage vector or into a vector from a certified EK2 host-vector system and propagated in *E. coli* K–12 under the appropriate containment conditions [see Section III–A–1–(a)–(5)]. Subsequently, this recombinant DNA may be returned to Host C and propagated there under P1 conditions. [43].

Containment levels for other classes of experiments involving non-HV1 systems may be expressly approved by the Director, NIH. (See Sections IV–E–1–b–(1)–(b), IV–E–1–b–(2)–(c), and IV–E–1–b–(3)–(b).)

N. *Modification of Section III–C–2, Invertebrate Host-Vector Systems*

Section III–C–2 of the Guidelines is replaced with the following modified text:

III–C–2. *Invertebrate Host-vector systems.*

III–C–2–a. *Insect viral vectors.* As soon as information becomes available on the host range restrictions and on the infectivity, persistence, and integration of the viral DNA in vertebrate and invertebrate cells, experiments involving the use of insect viruses to propagate DNA sequences will be evaluated by NIH on a case-by-case [45] and will be conducted under the recommended physical containment conditions. (See Section IV–E–1–b–(3)–(c)).

III–C–2–b. *Nonviral vectors.* Organelle, plasmid, and chromosomal DNAs may be used as vectors. DNa recombinants formed between such vectors and host DNA, when propagated only in that host (or a closely related strain of the same species), are exempt from thee Guidelines (see section I–E). DNa recombinants formed between such vectors and DNA from cells other than the host species require P1 physical containment for invertebrate cells in culture since invertebrate cells in culture inherently exhibit a very high level of containment. Experiments which require the use of whole animals will be evaluated by NIH on a case-by case basis [45].

O. *Addition of Section III–C–1–f, Vertebrate Host-Vector Systems—Non-Viral Vectors*

A new Section III–C–1–f is added to the Guidelines as follows:

III–C–1–f. *Nonviral vectors.* Organelle, plasmid, and chromosomal DNAs may be used as vectors. DNA recombinants formed between such vectors and host DNA, when propagated only in that host (or a closely related strain of the same species), are exempt from these Guidelines (see Section I–E). DNA recombinants formed between such vectors and nonviral DNA from cells other than the host species require only P1 physical containment for cells in culture since vertebrate cells in tissue culture inherently exhibit a very high level of containment. Recombinants involving viral DNA or experimetns which require the use of whole animals will be evaluated by NIH on a case-by-case basis [45].

P. *Addition of Section III–C–6, Return of DNA Segments to a Higher Eukaryotic Host of Origin*

A new Section III–C–6 is added to the Guidelines as follows:

III–C–6. *Return of DNA Segments to a Higher Eukaryotic Host of Origin.* DNA from a higher eukaryote (Host D) may be inserted into a lambdoid phage vector or into a vector from a certified EK2 host-vector system and propagated in *E. coli* K–12 under the appropriate containment conditions [see Section III–A–1]. Subsequently, this recombinant DNA may be returned to Host D and propagated under conditions of physical containment comparable to P1 and appropriate to the organism under study. [2A]

Dated: March 30, 1979.

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[FR Doc. 79–11037 Filed 4–10–79; 8:45 am]
BILLING CODE 4110–08–M